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Effect of Organic Solvent on the Electrochemical Immobilization of Glucose Oxidase into Polypyrrole Film

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INTRODUCTION

In the work of biosensor using enzymes, recent works have focused on the development of immobilized biological component. The attachment of the enzyme to the electrode can confer several advantages such as reusability of the sensor, stabilization of the enzyme and protection from proteolysis or thermal denaturation.[1] In the immobilization of the enzyme, the key point is stability of the enzyme and electrochemical coupling between the enzyme and host material.[2] For this target, many workers have investigated extensively the enzyme electrode,[3] and besides, they have made another attempt to improve amount of immobilized enzyme. Good examples of the method are immobilizing by gel matrix and cross-linking. These are actually successful in amount but the former brings a barrier for substrate diffusion and an enzyme may be damaged by the latter. It will come down to loss of enzyme activity.[5]

On the other hand, in the case of immobilizing of an enzyme in conducting polymer by electrosynthesis, the enzyme forms a coordinate bond with the polymer's backbone. However, because of intrinsic insulation and net chain of the enzyme, charge transfer and mass transport are obstructed during the film growing. For improve in immobilization, if adding of enzyme is followed up in the synthetic solution, the film growing will be dull. The film may not grow anymore. Therefore, it is not desirable to add more than proper amount of enzyme in synthetic solution.

In this paper, in the case of immobilizing of glucose oxidase (GOx) into polypyrrole (PPy), we investigated an effect of organic solvent on the electrochemical immobilization of GOx, and report qualitative evaluation on immobilization of GOx.

EXPERIMENTAL

PPy-GOx enzyme electrodes were obtained by electrosynthesis of the solution containing 0.2 mol dm^{-3} pyrrole (SIGMA), 0.1 mol dm^{-3} potassium chloride (ALDRICH) and 0.5 mg $M\ell^{-1}$ or 1.0 mg $M\ell^{-1}$ GOx (TYPE II, SIGMA). In some cases, a little amount of organic solvent (ethanol) was added. Synthetic potential of 800 mV vs. Ag/AgCl was applied for 300 mC cm^{-2}. Unless another comments, the potential refers to Ag/AgCl reference electrode.

Cyclic voltammetry was performed by potential sweep with ranging from –1.0 V to +0.5 V. The scan rate was 10 mV s^{-1} and 0.5 mol dm-3 potassium chloride electrolyte used. For evaluation on immobilization of GOx, ultraviolet spectroscopic analysis was performed using Hitachi 3501 spectrophotometer.

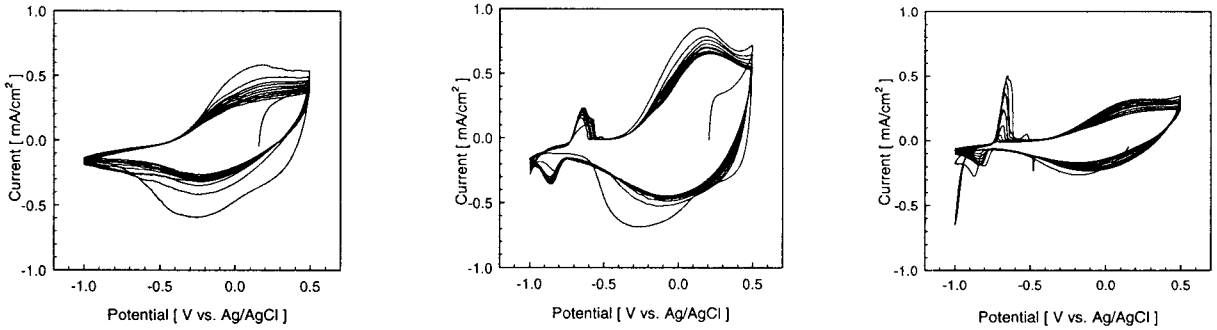
RESULTS AND DISCUSSTION

Fig. 1 describes cyclic voltammograms of PPy and PPy-GOx membrane. Fig. 1(a) shows typical redox curves of PPy. In the case of enzyme immobilized, new peaks were observed around –0.7 V as shown in Fig. 1(b) and Fig. 1(c). These new peaks reflect an influence of GOx on the redox behavior of the electrode. In addition, when ethanol was added, the influence was stronger. That suggest adding of ethanol has an effect on a condition of immobilizing of GOx.

Fig. 2 shows ultraviolet absorption spectra of synthetic solution before synthesis and after. As shown in Fig.

2(a), the peaks were observed for GOx at 275nm regardless of ethanol. In Fig. 2(b), the peaks were observed at 285nm and 335nm. The former correspond to GOx, the latter to residue pyrrole oligomer in the solution. We consider that the absorption peaks for GOx shift as many as 10nm because of applied potential. The peaks at 335nm reflect the influence of oligomer that is present as a residue after synthesis.

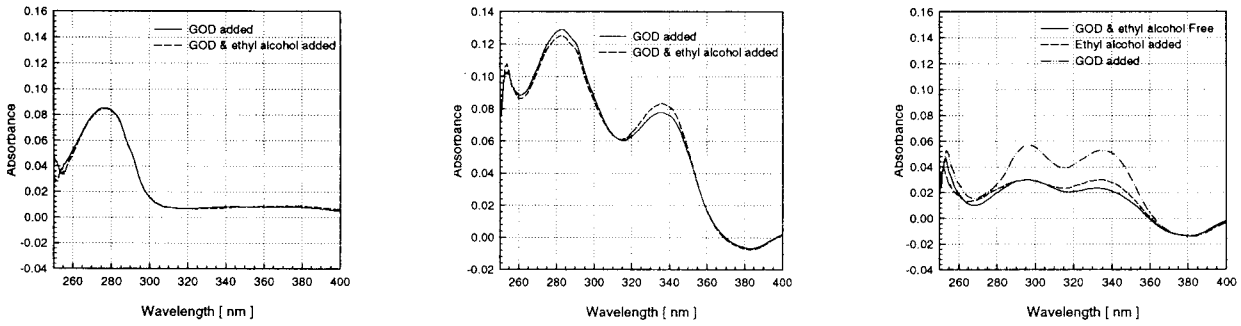
Another peaks at 295nm that correspond to oligomer are hidden by strong GOx peaks at 285nm. Fig. 2(c) shows the peaks for oligomer. Noteworthy thing in Fig. 2(b) is a peak at 285nm. When ethanol added in the synthetic solution, the intensity for remained GOx in the solution was weaker. That suggest the amount of immobilized



enzyme was increase in the case of ethanol added in the solution.

(a) (b) (c)

Fig. 1. Cyclic voltammograms of PPy and PPy-GOx enzyme electrodes. (a) PPy. (b) PPy-GOx prepared in the solution with 0.5 mg ml^{-1} GOx. (c) PPy-GOx prepared in the solution with 0.5 mg ml^{-1} GOx and 0.1 mol dm^{-3} ethanol.



(a) (b) (c)

Fig. 2. Ultraviolet spectra of synthetic solution before synthesis and after. (a) Before synthesis. Pyrrole, KCl and/or ethanol reference. (b) After synthesis. Pyrrole, KCl and/or ethanol reference. (c) After synthesis. Synthetic solution reference.

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